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## Suitability of Using Introduced *Hydrellia* spp. for Management of Monoecious *Hydrilla* *verticillata* (L.f.) Royle

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**PURPOSE:** The main objective of this study was to determine the suitability of using introduced hydrilla leaf-mining flies (*Hydrellia pakistanae* Deonier and *H. balciunasi* Bock) for the management of monoecious hydrilla (*Hydrilla verticillata* (L.f.) Royle). This was accomplished using a variety of procedures and experimental designs, including small container bioassays, development of a greenhouse-based fly colony reared exclusively on monoecious hydrilla, a larger tank study designed to evaluate short-term impact on both monoecious and dioecious hydrilla, use of small ponds to evaluate establishment in a more natural situation, evaluation of overwintering biology of the agents, and field releases to determine establishment success.

**BACKGROUND:** Introduction of biological control agents is a critical component of aquatic plant management. Using host-specific agents complements the goal of targeting nuisance vegetation while minimally affecting native vegetation. Two species of introduced ephydrid leaf-mining flies, *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock, have been shown to suppress dioecious hydrilla by reducing photosynthesis, thereby impacting biomass production, tuber formation, fragment viability, and hydrilla's ability to compete effectively with beneficial native vegetation (Doyle et al. 2002, 2005; Grodowitz et al. 2003; Owens et al. 2006, 2008). However, only limited research has been conducted evaluating their effectiveness on monoecious hydrilla found in the more northern portions of the United States. Dray and Center (1996) indicated that the flies would be highly suitable, but this research was confined mainly to short-term laboratory and greenhouse-based studies with no actual field releases; hence, more research is warranted.

## METHODS

**Bioassays:** Bioassays were conducted at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS, from February to June 2006. The experiment used small 3.5-L polycarbonate containers (18-cm diameter by 21-cm height) filled with 50 g wet weight (towel-blotted) hydrilla. Two biotypes of hydrilla were used: dioecious and monoecious. For each biotype, approximately 3 to 5 g of hydrilla containing 50 *Hydrellia pakistanae* eggs were added. The eggs were obtained from the ERDC greenhouse-based colonies. As fly emergence occurred, adults were enumerated and percent emergence determined. The number of days to first adult emergence, which is an indicator of developmental time, was also ascertained. The experiment was repeated seven

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times with one replication of each treatment. This was accomplished on separate dates and was used as the blocking factor. Blocking by date allowed a larger number of replications when fly numbers from research colonies were low. The data were analyzed using analysis of variance (ANOVA) in a randomized complete block design.

**Monoecious greenhouse-based colony:** In March 2007, a greenhouse-based colony of *H. pakistanae* developing solely on monoecious hydrilla was established at the ERDC. To initiate the colony, *H. pakistanae* immatures were obtained from the ERDC dioecious greenhouse colony. Rearing was accomplished using techniques described in Freedman et al. (2001). The colony was monitored through a total of 12 complete generations (Parental (P) Generation through the F11 Generation) until August 2008. Percent emergence and development time as a function of days to first emergence were determined. ANOVA was used to determine significant differences.

**Monoecious versus dioecious tank study:** This study was conducted in eleven 1,845-L fiberglass tanks located outdoors at the ERDC Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. Each tank contained 20 4-L pots (20.3 cm deep and 12.7 cm high) of dry heat sterilized substrate (electric soil sterilizer, Pro-Grow Supply Corp, Brookfield, WI) obtained from ponds at the LAERF. On 10 June 2008, each pot was planted with three 20-cm apical stem fragments of either monoecious or dioecious hydrilla. Treatments were as follows: five tanks contained monoecious hydrilla (two controls and three herbivores) and six tanks contained dioecious hydrilla (three control and three herbivores). The third monoecious control tank was planted, but was accidentally drained before any data were collected and was thus eliminated from the study. Alum-treated water from Lake Lewisville was added gradually as plants reached the surface, to a final height of 60 cm. An aquatic insecticide, Abate® 4-E (Abate) (Clarke Mosquito Control Products, Inc. Roselle, IL) was applied to control tanks weekly at 0.047 lb a.i./acre (1.5 fl. oz/acre). Four weeks after planting, approximately 14 to 16 dioecious hydrilla fragments, 10 to 20 cm in length, containing 200 immature (larvae & pupae) *Hydrellia* flies were added to each herbivore tank. After the initial fly introduction, ten 10-cm stems of hydrilla were collected from each tank every four weeks to enumerate *Hydrellia* spp. immatures per centimeter hydrilla and associated percent damaged leaves. Counts were determined using a stereo microscope at 7X to 10X magnification. Six pots per tank were randomly selected and harvested for tuber and turion numbers on 27 October 2008. ANOVA was used to determine significant differences.

**Small pond study:** Three 6-m by 6-m by 1-m-deep, lined ponds at the LAERF were used for *Hydrellia* fly rearing on monoecious hydrilla. One pond in 2006 and two ponds in 2007 were inoculated with monoecious hydrilla tubers and turions throughout the growing season as available. Tubers and turions were harvested from monoecious hydrilla grown in 1,845-L fiberglass tanks located outdoors at the LAERF. Fly colonization was allowed to proceed naturally from populations in nearby ponds as well as a single release in 2007 of a small quantity of flies (approximately 1,200 immatures split equally across all ponds) obtained from a monoecious greenhouse-based colony at the ERDC. At periods throughout the growing season in 2007, number of fly immatures and associated damage were evaluated in each pond by randomly collecting approximately 10-20 hydrilla sprigs and enumerating immatures per centimeter and associated percent damaged leaves. Counts were determined using a stereo microscope at 7X to 10X magnification. ANOVA was used to determine significant differences.

In addition, the relative number of adult *Hydrellia* flies per pond was quantified using a modified soap-dish method. Soap decreases water's surface tension, causing insects landing on its surface to sink and drown. On 3 October 2008, one container filled with a weak soap solution was floated in each of the three monoecious ponds and three randomly selected dioecious ponds nearby for approximately 24 hr. Numbers of adult *Hydrellia* spp. were enumerated and ANOVA was used to determine significant differences.

**Fly overwintering:** Overwintering biology was qualitatively examined at the LAERF during the winter months of 2005-2006 using several earthen ponds. Monthly samples of hydrilla were collected and leaves examined microscopically for larval *Hydrellia*. In addition, hydrilla and organic debris from pond edges (i.e., leaves and other plant material) were collected monthly and placed in Berlese funnels to determine presence of adults or larvae (Berlese extraction technique). Hydrilla stems were dissected if they exhibited signs of tunneling damage, in the form of darkened, longitudinal patches of stem. Since larval stages cannot be used to accurately differentiate *H. pakistanae* from other *Hydrellia* spp., hydrilla stems were placed in water-filled containers in an attempt to rear adult flies for positive identification.

Three rearing methods were used, including 1) larvae were removed from original stems and placed in Petri dishes with fresh hydrilla, 2) stems were dissected to locate larvae, gently closed, and then submersed in Petri dishes, and 3) stems (regardless of signs of tunneling) with undamaged leaves were placed in a mason jar fitted with a mesh top and filled with water. All three rearing methods were attempted in the laboratory at room temperature (~22 °C) with 14:10 photoperiod.

**Fly release methodologies:** Hydrilla containing leaf-mining flies, *H. pakistanae* and *H. balciunasi*, with the former being the dominant species, was used to introduce the insects into Lake Gaston. Insects used for release were obtained mainly from the LAERF, though minimal numbers were also obtained from small cement ponds at ERDC, Vicksburg, MS. For the original 2004 releases, insects were reared on dioecious hydrilla cultured in a series of 0.2-ha to 0.3-ha ponds using water obtained directly from Lewisville Lake (Harms et al. 2009). Ponds were fertilized with ammonium sulfate as needed to promote vigorous growth of hydrilla. Immature flies were hand-collected by gathering surface canopy biomass from the hydrilla ponds. The biomass containing *Hydrellia* larvae and pupae was packed loosely into ice chests, and shipped overnight to Lake Gaston. Release sites were selected based on minimal outside disturbance due to human activities and low probability of future herbicide applications. Hydrilla remained canopied in these sites for the majority of the growing season. Two sites were chosen to allow for higher numbers of stockings in a limited area with the expectation that these areas will be used as future nursery areas for other areas on the lake (Figure 1).

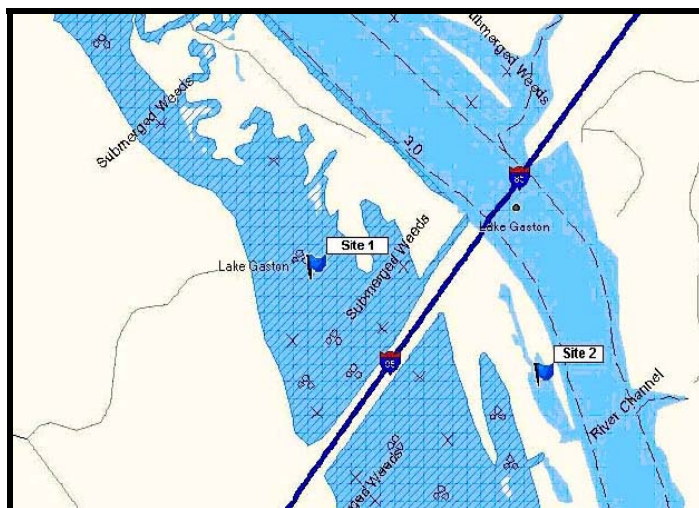


Figure 1. Two hydrilla leaf-mining fly release sites were selected for biocontrol evaluations in Lake Gaston, NC/VA.

For the original 2004 releases, hydrilla containing immature flies was released directly into the lake. This practice was discontinued after concerns were raised about introducing dioecious hydrilla to Lake Gaston where the dioecious biotype is thought to be limited and the monoecious biotype is predominant (Ryan et al. 1996).

Following the original releases, dioecious hydrilla from LAERF was released on Lake Gaston into cages measuring 1 m by 1 m, constructed from 2-in. PVC pipe, and covered with 0.6-cm black mesh (Figure 2). Cages were constructed in order to prevent dispersal of dioecious hydrilla into the lake while at the same time enabling flies to move out of the contained area. Hydrilla was left in cages for three weeks or less to prevent establishment of new dioecious hydrilla populations from the formation and release of turions.

Beginning in 2007, monoecious hydrilla containing *Hydrellia* immatures was released directly into the lake at the two sites identified previously (Figure 1). Monoecious hydrilla supporting flies was obtained either from the ERDC greenhouse-based monoecious colony or from monoecious rearing ponds at LAERF.

Three sampling protocols were used to assess establishment success:

- **Stem sampling:** The first involved random collection of approximately 2 kg wet weight of hydrilla from three to six areas throughout the two release sites. Hydrilla was shipped to ERDC where 20 to 30 stem pieces ranging in length from 10 cm to 20 cm were chosen at random, weighed, stem length measured, and number of immatures and associated fly-damaged leaves counted using a stereo microscope at 7 X to 10 X magnification.
- **Berlese funnel extraction:** In addition, approximately one half of each hydrilla sample (~1 kg) was placed into Berlese funnels to extract larvae. The Berlese funnels use heat from light bulbs to slowly dry the plant material, thereby forcing the larvae further into the bottom of the funnel where they eventually fall into a jar containing 70 percent alcohol for later enumeration.
- **Point sampling:** In addition, on two occasions, a point intercept sampling method was used. During each sampling period, a series of points arranged in a grid were sampled for the presence or absence of the hydrilla leaf-mining flies. At each sampling point, a single stem was collected and processed as mentioned previously. Number of points varied depending on the area sampled and, for example, ranged from about 50 points for Site 1 sampled in August 2006 to over 2000 points during the initial whole-lake sampling accomplished in September 2005.

**Statistical Analysis.** All statistical analyses were accomplished using Statistica version 8.0 (StatSoft, Inc., 2007, Tulsa, OK). Unless otherwise indicated the alpha-level was 0.05. Post-hoc comparisons were made using a Newman-Keuls test.



Figure 2. Hydrilla containment cages stocked with fly-infested dioecious hydrilla were deployed for fly release in Lake Gaston, NC/VA beginning in 2005.



## RESULTS AND DISCUSSION

**Bioassays:** Bioassays of *H. pakistanae* flies reared on monoecious hydrilla exhibited lower survival with no statistical differences noted for developmental time (Figure 3). Percent emergence was almost two-fold lower for those reared entirely on the monoecious biotype (33 percent emergence for monoecious as opposed to 55 percent for the dioecious biotype). While no statistical differences were noted for days to first emergence, the mean days to first emergence value was almost one day shorter for the dioecious biotype relative to that observed for the monoecious biotype. These differences were unexpected based on high suitability of the monoecious biotype reported by Dray and Center (1996).

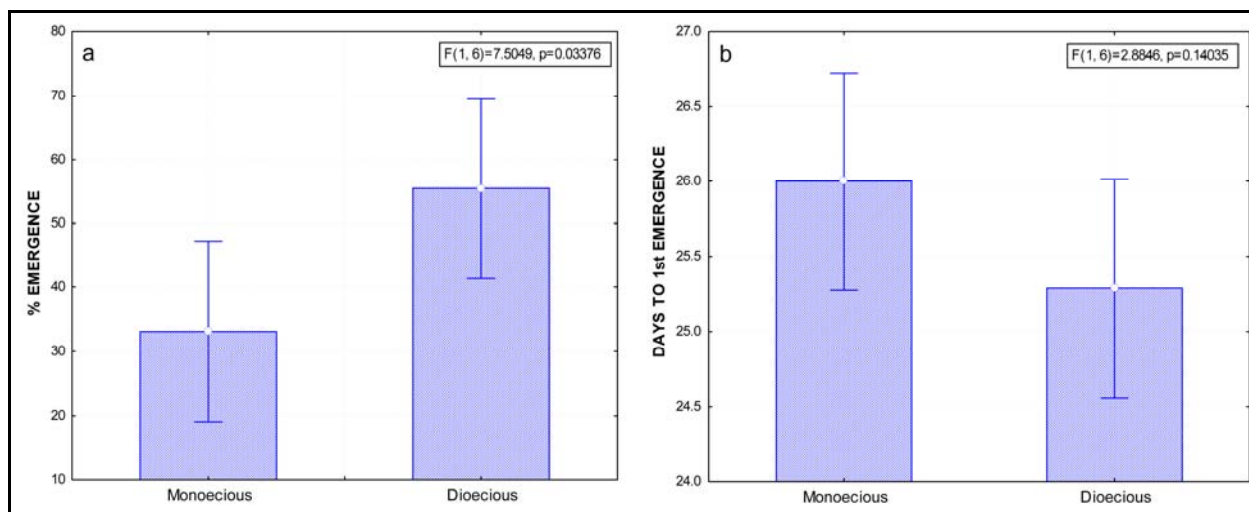


Figure 3. Comparison of percent emergence (a) and days to first adult emergence (b) for *H. pakistanae* on monoecious and dioecious hydrilla grown in a greenhouse at the ERDC in Vicksburg, MS. One-way ANOVA statistics are shown for each graph. Bars represent the 95-percent confidence interval as determined by the pooled error component.

**Monoecious greenhouse-based colony:** Results observed for the bioassay experiment were similar to those observed for the monoecious greenhouse-based colony. *Hydrellia pakistanae* survival, as indicated by percent emergence, was lower for flies reared through 11 generations on the monoecious biotype than was observed by Freedman et al. (2001) on dioecious colonies (Figure 4a). *Hydrellia pakistanae* percent emergence for all 11 generations was  $36.5 \pm 2.5$  SE and 49 percent for monoecious and dioecious colonies (based on data from Freedman et al. 2001), respectively. Of the 12 generations reared (including parental) on monoecious hydrilla, eight generations (67 percent of the total) had mean emergence values less than 50 percent.

Increased developmental time was also observed for the flies reared on the monoecious hydrilla (Figure 4b). Under similar conditions, mean number of days to first adult emergence was  $29.97 \pm 0.51$  SE regardless of generation for flies reared on monoecious hydrilla compared to only 21 days when reared on the dioecious biotype (Freedman et al. 2001); a difference of approximately 9 days. All monoecious biotype generations with the exception of the F2 generation exhibited over 25 days to first emergence. Previous research has shown that increased developmental time in *H. pakistanae* is related to plant nutritional status and often correlated to lowered fecundity (Wheeler

and Center 1996; Grodowitz et al. 2003). Because of the observed differences in emergence success and development time between the two biotypes, nutritional differences are suspected and more research on nutritional status should be conducted.

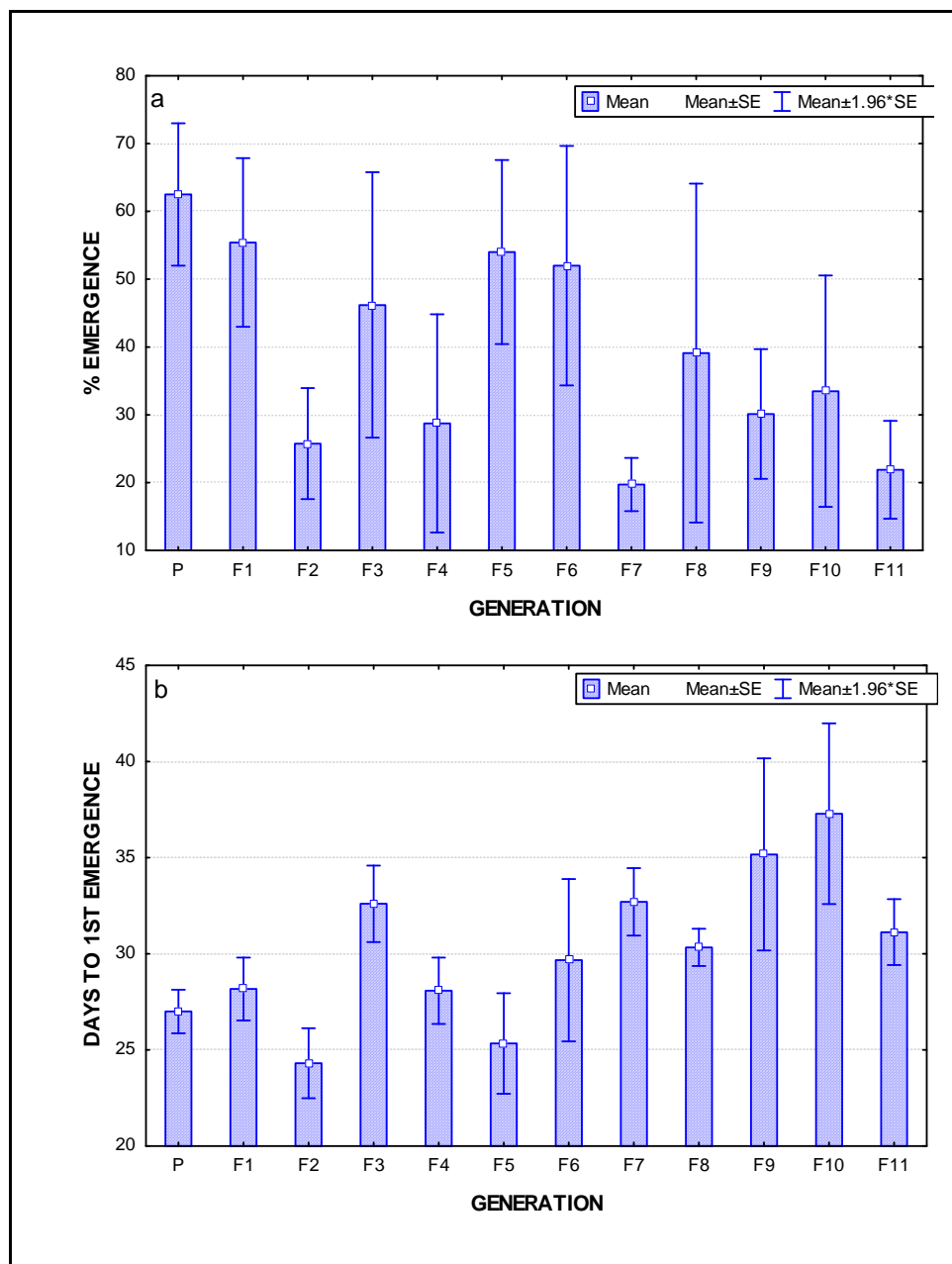


Figure 4. Percent emergence (a) and days to first emergence (b) for the monoecious, greenhouse-based colony of hydrilla leaf-mining flies (*H. pakistanae*) reared at ERDC in Vicksburg, MS. Bars represent the 95 percent confidence interval. Significant difference between generations for percent emergence was detected using ANOVA at  $f(11, 86) = 3.4$ ,  $p = 0.0007$ , and for days to first emergence  $f(11, 86) = 8.8$ ,  $p < 0.0000$ .

**Monoecious versus dioecious tank study:** Significant differences were noted in colonization of *Hydrellia* flies and percent hydrilla leaf damage between hydrilla biotypes (Figure 5). Fly stocking rates were equal between biotypes, yet four weeks after release, fly levels in dioecious tanks were 5.3-fold higher than monoecious on 5 August 2008 (Figure 5a). For all remaining sampling dates (with the exception of 9/30/08), fly levels were not significantly different between hydrilla biotypes (Figure 5a). Initially, percent leaf damage in dioecious tanks was greater than monoecious by 2.4-fold (Figure 5b). Average difference in percent leaf damage between biotypes ranged from approximately 2 percent (9/2/2008) to 20 percent (8/5/2008), with significant differences between biotypes occurring in all but the 9/2/2008 samples (Figure 5b).

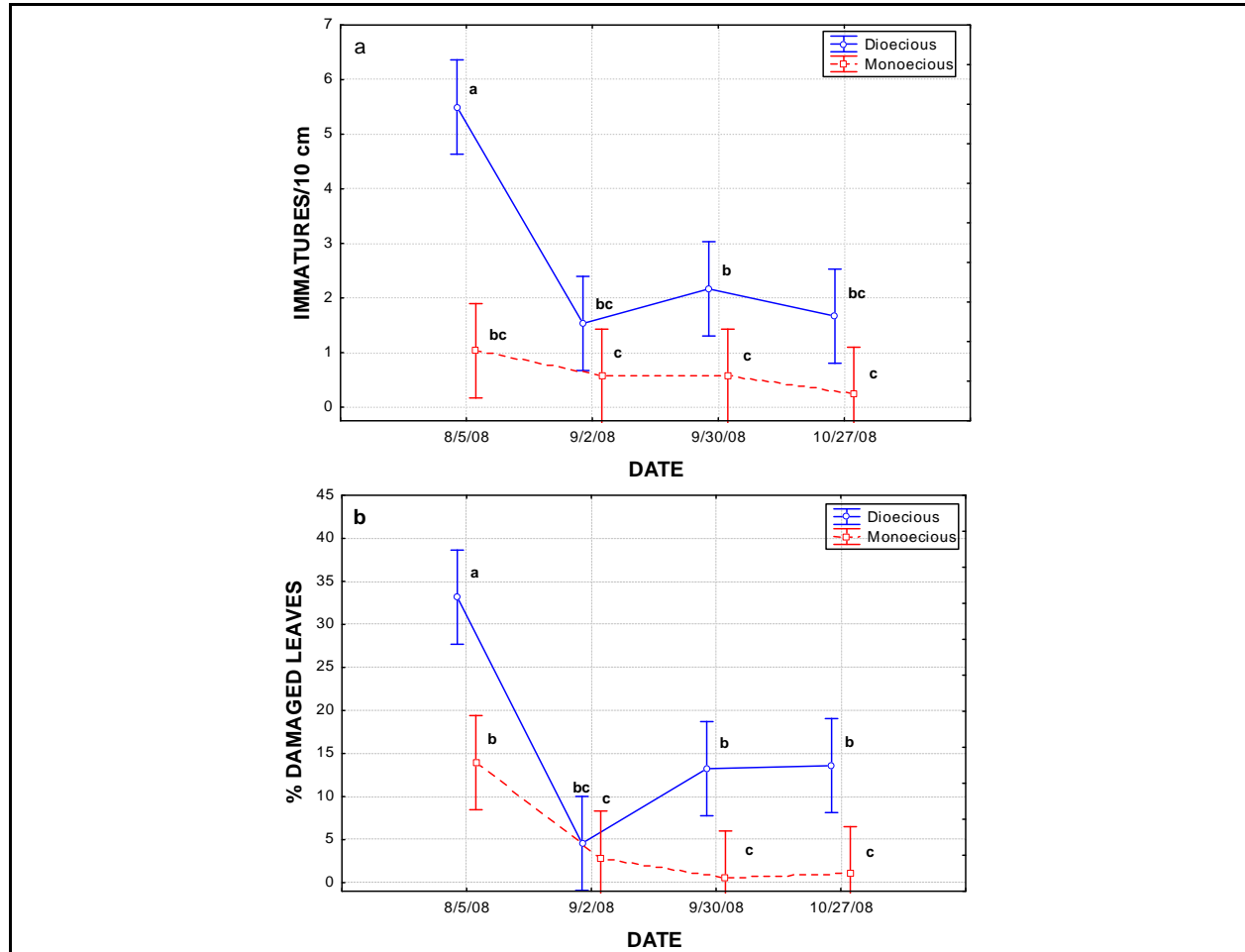


Figure 5. **(a)** Mean ( $\pm$  0.95 confidence interval) *Hydrellia* spp. per collection date for monoecious and dioecious hydrilla biotypes reared in fiberglass tanks in Lewisville, TX in 2008. Means with the same letter are not significantly different (Newman-Keuls multiple range test,  $\alpha = 0.05$ ). Two-way analysis of variance, date:  $p < 0.0000$ ,  $F = 12.299$ ,  $DF = 3, 232$ ; biotype:  $p < 0.0000$ ,  $F = 46.650$ ,  $DF = 1, 232$ ; interaction:  $p = 0.0003$ ,  $F = 6.576$ ,  $DF = 3, 232$ . **(b)** Mean ( $\pm$  0.95 confidence interval) percent damaged leaves per collection date for monoecious and dioecious hydrilla biotypes reared in fiberglass tanks in Lewisville, TX in 2008. Means with the same letter are not significantly different (Newman-Keuls multiple range test,  $\alpha = 0.05$ ). Two-way analysis of variance, date:  $p < 0.0000$ ,  $F = 20.777$ ,  $DF = 3, 232$ ; biotype:  $p < 0.0000$ ,  $F = 34.689$ ,  $DF = 1, 232$ ; interaction:  $p = 0.0177$ ,  $F = 3.433$ ,  $DF = 3, 232$ .



Tuber production of neither biotype was impacted by *Hydrellia* fly presence (Figure 6a); yet significantly lower turion numbers were observed for monoecious plants impacted by *Hydrellia* spp. (Figure 6b). Lower production of turions could not be attributed solely to *Hydrellia* feeding. During the course of the experiment, plants of both biotypes were unintentionally infested with *Paraponyx diminutalis* Snellen, the Asian hydrilla moth (Buckingham and Bennett 1996). This species appeared to be established in monoecious plants, with higher numbers and damage observed qualitatively. While decreases in turion numbers were caused by herbivore feeding, this study could not distinguish whether differences in turion number was due to *Hydrellia* flies or *Paraponyx*. It may be beneficial to conduct additional studies to determine if *P. diminutalis* has a preference for the monoecious biotype.

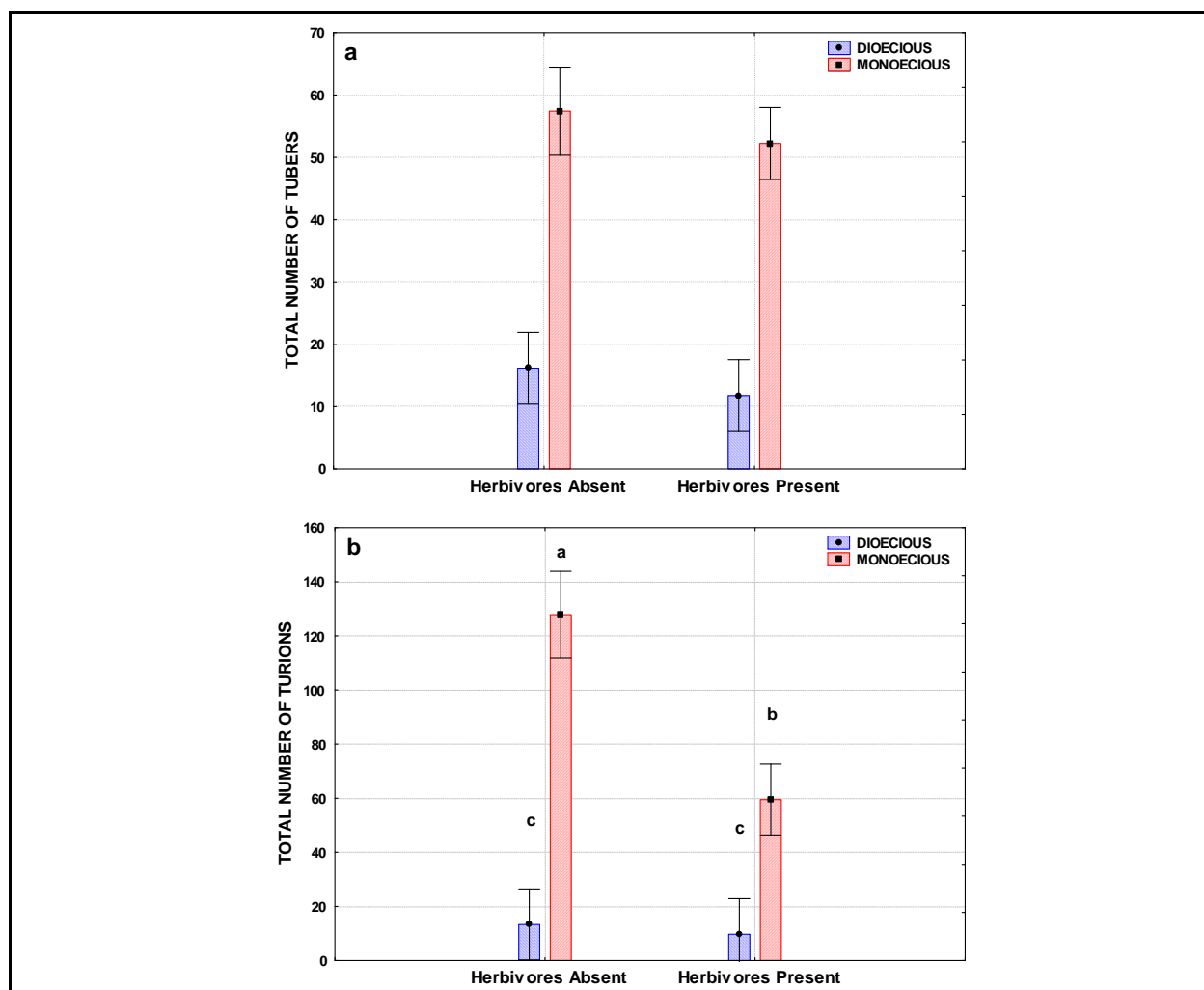


Figure 6. **(a)** Mean ( $\pm$  0.95 confidence interval) number of tubers per treatment and hydrilla biotype. Tubers were harvested from fiberglass tanks in Lewisville, Texas on 27 October 2008. Two-way ANOVA, treatment:  $p = 0.1224$ ,  $F = 2.452$ ,  $DF = 1, 62$ ; biotype:  $p < 0.0000$ ,  $F = 178.210$ ,  $DF = 1, 62$ ; interaction:  $p = 0.8957$ ,  $F = 0.017$ ,  $DF = 1, 62$ . **(b)** Mean ( $\pm$  0.95 confidence interval) number of turions per treatment and hydrilla biotype. Turions were harvested from fiberglass tanks in Lewisville, Texas on 27 October 2008. Means with the same letter are not significantly different (Newman-Keuls multiple range test,  $\alpha = 0.05$ ). Two-way ANOVA, treatment:  $p < 0.0000$ ,  $F = 26.718$ ,  $DF = 1, 62$ ; biotype:  $p < 0.0000$ ,  $F = 139.307$ ,  $DF = 1, 62$ ; interaction:  $p < 0.0000$ ,  $F = 21.623$ ,  $DF = 1, 62$ .

**Small pond study:** Even with high numbers of *Hydrellia* in nearby dioecious ponds serving as a source for colonization, counts of immature flies were significantly lower in monoecious ponds compared with dioecious ponds throughout the 2008 growing season (Figure 7). For example, 57-fold and 49-fold higher numbers of immatures were noted for the dioecious biotype for July and August 2008. In addition, number of immatures for the monoecious biotype remained low for the entire sampling period, never exceeding 0.22 immatures/10 cm. At the LAERF, flies typically colonize new hydrilla ponds naturally. For example, in an ongoing study begun in 2006 at the LAERF, approximately one month after planting ponds with dioecious hydrilla, *Hydrellia* flies naturally colonized hydrilla with levels reaching 5.25 immatures /10 cm (unpublished data).

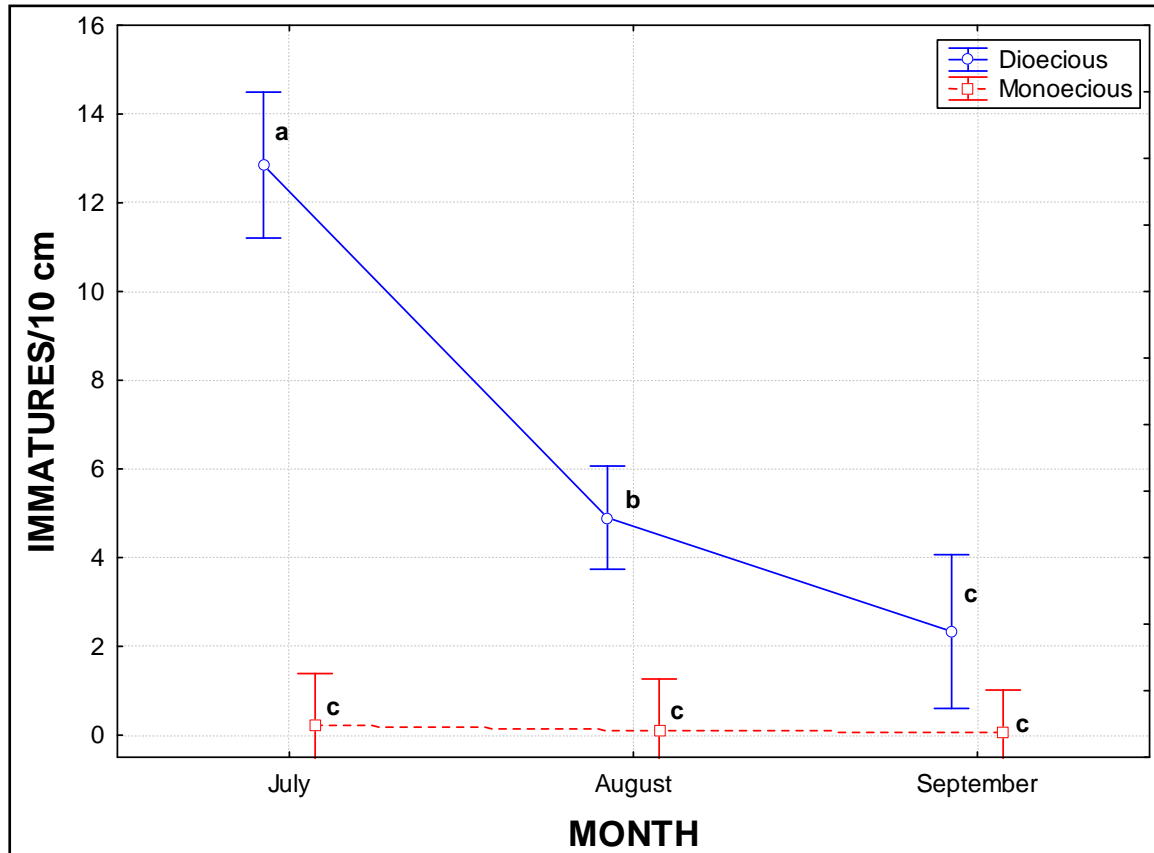


Figure 7. Mean ( $\pm$  0.95 confidence interval) number of *H. pakistanae* immatures/10 cm hydrilla stem fragment through time for both monoecious and dioecious hydrilla biotypes during 2008 at Lewisville, TX. Two-way ANOVA, month:  $p < 0.0000$ ,  $F = 31.05$ ,  $DF = 2, 103$ ; biotype:  $p < 0.0000$ ,  $F = 142.99$ ,  $DF = 1, 103$ ; interaction:  $p < 0.0000$ ,  $F = 200.92$ ,  $DF = 2, 103$ .

These differences were apparently not caused by the adult flies' lack of attraction to the monoecious biotype. Numbers of adults captured using soap dishes (i.e., a rough, relative estimate of adult fly visits), did not differ between the two biotypes over a 24-hr period in October 2008 (Figure 8). Average number of introduced *Hydrellia* spp. adults collected from monoecious ponds was 337 compared to 235 for the dioecious ponds. Hence, it appears that the differences in immature numbers in the monoecious biotype ponds must have been due to lowered immature survival and/or limited oviposition by adult females. It has been observed at the LAERF and at field sites that the monoecious biotype tends to remain below the water's surface during the growing season, while

dioecious hydrilla tends to produce a dense, topped-out canopy throughout the season (unpublished data). It is not clear whether this results in less oviposition. More research is warranted on oviposition behavior.

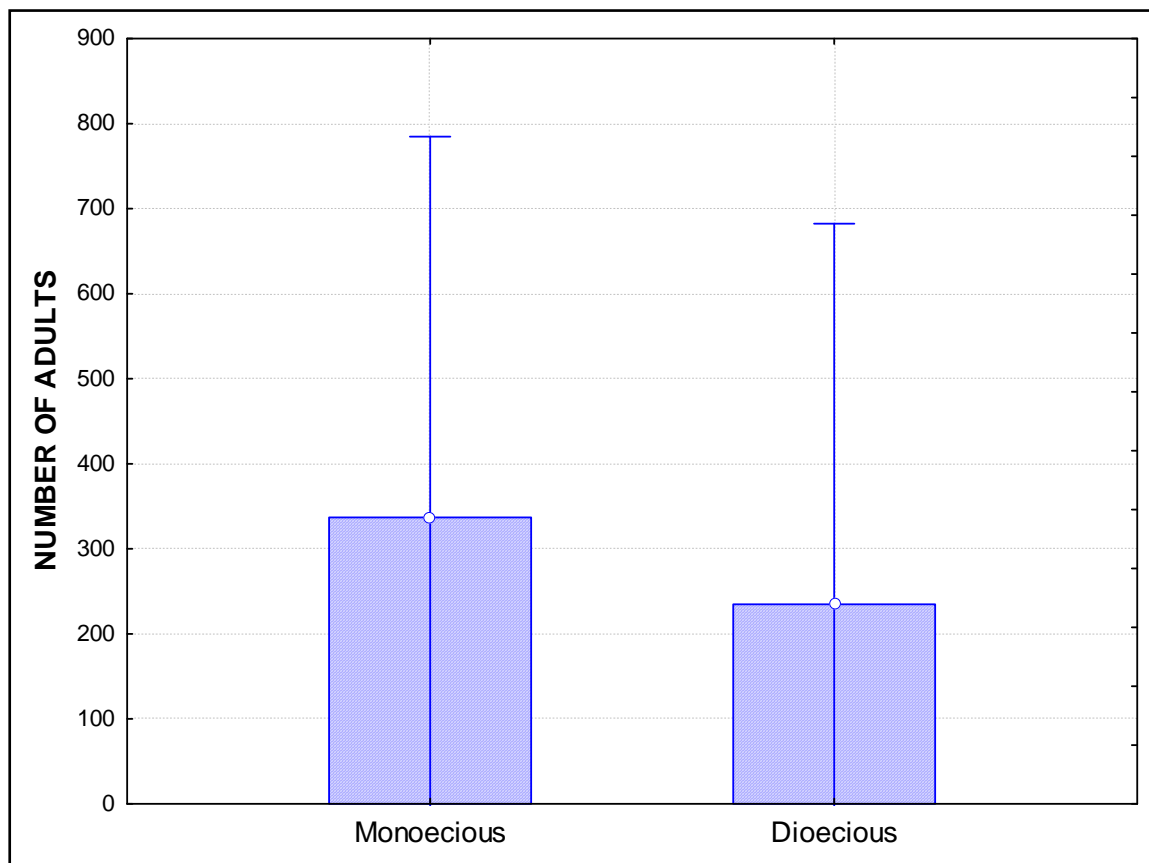


Figure 8. Number of adult *H. pakistanae* captured in soap dishes over a 24-hr period on October 3 through October 4, 2008 in Lewisville, TX for ponds containing either monoecious or dioecious hydrilla. One-way ANOVA indicated no significant differences at  $p = 0.68$ ,  $F = 0.20$ ,  $DF = 1, 4$ .

**Fly overwintering:** Based on numerous examinations of dioecious hydrilla during the winter at the LAERF, it was found that flies overwinter as larvae within stems (Harms and Grodowitz, in review). Larvae evidently move from leaves and enter stems, presumably before winter senescence of plants. The flies remain within stems until resumption of growth in spring, when they emerge as adults directly from stems or move into the leaves to complete development. During the winter, development is slowed considerably due to lower temperatures, with larvae appearing lethargic while in the stems. No adults or eggs were found during winter collections.

This overwintering strategy has important implications for the ability of *Hydrellia* spp. larvae to overwinter in the monoecious biotype at Lake Gaston. For dioecious hydrilla, although winter senescence does typically occur, there usually remains appreciable aboveground biomass that can support overwintering larvae. However, for the monoecious hydrilla biotype found at Lake Gaston, recent studies and qualitative observations on Lake Gaston have indicated that there is a complete dieback of aboveground biomass that results in unavailability of overwintering material, thereby precluding sustainable establishment since the flies would not be able to survive the winter.

However, recent observations of monoecious hydrilla cultured outdoors at the LAERF in small lined ponds indicate that appreciable quantities of the monoecious biotype can remain throughout the winter under the water surface or as floating mats providing suitable habitat for fly overwintering. The extent of this occurring on Lake Gaston is unknown and more research on overwintering of the monoecious hydrilla and its impact on *Hydrellia* spp. is needed.

**Fly release and establishment:** Over 1.8 million flies, mainly immature *H. pakistanae* and to a lesser extent *H. balciunasi*, were released at two sites directly on Lake Gaston beginning in September 2004 and continuing through October 2008 (Table 1). More than 1,000,000 flies were released at Site 1 with the remaining 700,000 or so released at Site 2 through October 2008. In most cases, flies released were healthy with no obvious signs of stress due to the shipment. However, during 2006 it was noted that larvae in shipments sent during late August and September were stressed, likely due to elevated temperatures within shipping containers. Stress was indicated by languid larvae showing limited movement under stimulation. In addition, many larvae were flattened and flaccid, indicating a loss of turgor pressure common in unhealthy larvae. To avoid temperature stress with future shipments, ice packs were added to the bottom of shipping containers and then covered with layers of insulation to avoid freezing the hydrilla in order to maintain internal temperatures at more reasonable levels.

<b>Table 1. Hydrilla leaf-mining flies (<i>H. pakistanae</i>) released in Lake Gaston NC/VA at two sites between September 2004 and October 2008. Two methods of release were used. In most cases <i>Hydrellia</i> spp. contained within dioecious hydrilla were released into cages to prevent contamination of what is mainly monoecious hydrilla on Lake Gaston. <i>Hydrellia</i> spp. contained within monoecious hydrilla were released directly into the lake.</b>				
Date	Number Released	Site 1	Site 2	Type of Release/Biotype
14 September 2004	600,000	0	600,000	Direct/Dioecious
17 September 2005	720,000	720,000	0	Cages/Dioecious
23 August 2006	145,422	96,948	48,474	Cages/Dioecious
8 September 2006	152,256	101,504	50,752	Cages/Dioecious
16 October 2006	122,550	81,700	40,850	Cages/Dioecious
<b>Total dioecious</b>	<b>1,740,228</b>	<b>1,000,152</b>	<b>740,076</b>	
25 September 2007	5,377	5,377	0	Direct/Monoecious
26 August 2008	8,000	8,000	0	Direct/Monoecious
22 September 2008	31,818	31,818	0	Direct/Monoecious
16 October 2008	57,669	57,669	0	Direct/Monoecious
<b>Total monoecious</b>	<b>102,864</b>	<b>102,864</b>	<b>0</b>	
<b>Total flies released</b>	<b>1,843,092</b>	<b>1,103,016</b>	<b>740,076</b>	

Establishment of *Hydrellia* spp. flies on Lake Gaston is tentative at best with only very minimum evidence in the form of leaf damage and presence of immatures or adults. No evidence of establishment was recorded during 2005 by the point sampling, stem sampling, or Berlese funnel extraction techniques. This is not unexpected, given the short two-year period during which actual releases were made. Confirmed establishment for water bodies in the southeast in the 1990's typically took longer than five years before significant signs of damage and associated immatures and/or adults were observed consistently (Center et al. 1997; Grodowitz et al. 1997, 2003; and unpublished data). Reasons for this are not fully understood but are probably related to fly dispersal

after emergence, location of establishment foci, and/or selection of individuals suitable for the new climatic regime.

However, on more than one occasion during 2006, evidence of establishment on Lake Gaston was observed. During stem sampling on 17 October 2006, one stem from Site 2 exhibited over 30 percent leaf damage and supported three *Hydrellia* pupae, both of which are encouraging signs of establishment. In addition, during 23 and 24 August 2006 sampling, two larvae were observed in one replication of the Berlese extractions. This observation occurred prior to releases that year, indicating that these individuals originated from in-lake breeding, thus providing tentative evidence for overwintering and permanent establishment. While numbers of immatures and adults were low or non-existent for the remaining sampling periods during 2006, Berlese funnel extractions recovered additional individuals, with highest counts occurring during October 2006 at Site 2 where > 20 immatures/kg were recorded. In addition, a single adult female *H. pakistanae* was recovered from Berlese extractions during November 2006 at Site 1.

During 2008, additional observations of immatures and associated damage were conducted using both Berlese funnel extractions and stem sampling. In September 2008, 21 (8 percent) of the 240 stems examined had visible signs of fly larvae feeding damage, ranging from 1 to >15 percent leaf damage. This observation represented the highest leaf damage recorded to date. In addition, two stems from the August 2008 sample exhibited leaf damage and contained 21 immatures/kg; while this number is considered low, it showed the introduced flies were reproducing and possibly establishing on monoecious hydrilla in Lake Gaston. Although overall evidence to date for fly establishment is minimal and at best tentative, these observations are encouraging and additional monitoring is warranted.

**SUMMARY:** Experiments and field studies conducted since 2004 indicate that monoecious hydrilla is not as suitable a host for introduced *Hydrellia* spp. as is dioecious hydrilla found elsewhere in the United States. This conclusion is based in part on reduced survival and longer developmental time in bioassay experiments and greenhouse colony rearing as well as lower colonization success and subsequent low population growth rates in larger outdoor systems. Additionally, lack of long-term establishment at Lake Gaston field sites appears to indicate poor suitability of the monoecious biotype as a host plant. This was not to be expected. Research conducted earlier on the use of flies on monoecious hydrilla indicated that while developmental time is longer, survival was as good as (if not better than) that observed on the dioecious biotype (Dray and Center 1996).

Although most evidence points to low suitability of the monoecious biotype to support flies, surveys should be continued on Lake Gaston to examine whether the flies have established. Studies examining differences in nutritional composition of the monoecious biotype as it relates to fly development and success should be conducted to help pinpoint observed differences in fly developmental time, survival, and percent emergence. In addition, research should be conducted examining fly oviposition behavior in relation to monoecious biotype growth characteristics.

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